A Point Mutation of Tyr-759 in Interleukin 6 Family Cytokine Receptor Subunit gp130 Causes Autoimmune Arthritis

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Abstract

We generated a mouse line in which the src homology 2 domain–bearing protein tyrosine phosphatase (SHP)-2 binding site of gp130, tyrosine 759, was mutated to phenylalanine (gp130F759/F759). The gp130F759/F759 mice developed rheumatoid arthritis (RA)-like joint disease. The disease was accompanied by autoantibody production and accumulated memory/activated T cells and myeloid cells. Before the disease onset, the T cells were hyperresponsive and thymic selection and peripheral clonal deletion were impaired. The inhibitory effect of IL-6 on Fas ligand expression during activation-induced cell death (AICD) was augmented in gp130F759/F759 T cells in a manner dependent on the tyrosine residues of gp130 required for signal transducer and activator of transcription 3 activation. Finally, we showed that disease development was dependent on lymphocytes. These results provide evidence that a point mutation of a cytokine receptor has the potential to induce autoimmune disease.

Key words: IL-6 • rheumatoid arthritis • gp130 • SHP-2 • STAT-3

Introduction

Rheumatoid arthritis (RA)* is a frequent autoimmune disorder; however, its etiology and pathogenesis are still not completely understood (1, 2). Genetic background and environmental factors, such as bacterial or viral infection, are thought to be involved in its onset. The genetic linkage of RA to an MHC class II molecule (HLA-DR) and the infiltration of T cells into the synovium of RA joints suggest that T cells are involved in the abnormal immune responses associated with RA (1). Proinflammatory cytokines, such as TNF-α, IL-1, and IL-6 are thought to play crucial roles in the pathology of RA and autoimmune diseases (3–5). Several engineered mutant mice that spontaneously develop arthritis have been reported. Analyses of the K/BxN TCR transgenic mouse showed that an autoantibody specific to a ubiquitous molecule can induce an organ-specific autoimmune disease (2, 6, 7). The HTLV-1 transgenic mouse is an animal model of arthritis that is triggered by viral infection (8). TNF-α transgenic mouse and TNF AU-rich elements-deficient mice revealed that TNF-α is a pivotal cytokine for the induction of other proinflammatory cytokines and for the abnormal growth of synovial cells (9, 10). IL-1α transgenic mice (11) and gene targeting of an IL-1 receptor antagonist (12) indicated that the increased

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*Abbreviations used in this paper: AICD, activation-induced cell death; RA, rheumatoid arthritis; RAG, recombination activation gene; RF, rheumatoid factor; SEB, staphylococcal enterotoxin B; SHP, src homology 2 domain–bearing protein tyrosine phosphatase; SOCS, suppressor of cytokine signaling; STAT, signal transducer and activator of transcription; TRAP, tartrate-resistant acid phosphatase.
activity of a proinflammatory cytokine (IL-1) results in arthritis. The generation of RA-like joint diseases in a variety of engineered mutant mice may reflect the heterogeneity of human RA or the complicated mechanisms underlying the disease.

IL-6, which was originally identified as a B cell differentiation factor (13, 14), is now known to be a multifunctional cytokine that regulates the immune response, hematopoiesis, the acute phase response, and inflammation (4, 15). The first suggestion that IL-6 is involved in autoimmunity came from the finding that it is produced by cardiac myxoma cells, and patients with cardiac myxoma frequently show autoimmune symptoms (16). Since then, several pieces of evidence have been reported that suggest the involvement of IL-6 in autoimmune diseases, including RA (4, 17). Furthermore, IL-6 is required for experimentally induced autoimmune diseases or autoimmunity, including type II collagen- and antigen-induced arthritis (18–20), myelin oligodendrocyte protein-induced experimental autoimmune encephalomyelitis (21, 22), and pristane-induced autoantibody production (23). These results suggest that IL-6-dependent signaling pathways are involved in the pathogenesis of these experimentally induced autoimmune diseases. However, it is unknown whether IL-6 and its receptor system are involved in the pathogenesis of spontaneously occurring autoimmune diseases. The IL-6 receptor consists of an α chain and the subunit gp130. gp130 is shared by the receptors for IL-6 family cytokines, including leukemia inhibitory factor, ciliary neurotropic factor, oncostatin M, IL-11, and cardiotoxin–1 (24). Binding of one of these cytokines to its receptor and gp130 activates JAK-1, JAK-2, and TYK-2, which phosphorylate tyrosine residues in the cytoplasmic domain of gp130. The transcription factor signal transducer and activator of transcription (STAT)-3 is recruited to the phosphorylated tyrosine residues in the YXXQ motif of gp130, where it is activated and dimerized, and subsequently enters the nucleus and regulates gene expression (24, 25). The src homology 2 domain–bearing protein tyrosine phosphatase (SHP)-2, is recruited to the phosphorylated tyrosine residue Y759 in gp130, where it becomes activated and forms a complex with the adaptor docking proteins, Gab1 and Gab2, leading to activation of the Ras–MAP kinase pathway (26). Several experiments in vitro have indicated that the SHP-2- and STAT-3–mediated signal-transduction pathways that are initiated through gp130 are involved in growth, differentiation, and gene expression in various cell lines (24, 25, 27, 28). SHP-2–mediated ERK mitogen-activated protein (MAP) kinase activation has been suggested to play negative roles in STAT-3–mediated biological responses (29, 30). Tyrosine residue Y759 also provides the binding site for the suppressor of cytokine signaling (SOCS)-3 protein, which negatively regulates the gp130 signals (31, 32). Recently, we generated a series of knock-in mouse lines in which the gp130-mediated SHP-2 or STAT-3 signals are selectively disrupted. To make the SHP-2 signal-deficient mice (gp130F759/F759), we mutated Y759 of gp130 to phenylalanine (F759). The gp130F759/F759 mice are born normal but develop splenomegaly, lymphadenopathy, and an enhanced acute phase reaction. In contrast, the STAT-3 signal-deficient mice (gp130O/F759) die perinatally, as do gp130–deficient mice (gp130+/−). The embryonic fibroblasts from the gp130F759/F759 mice show a severe reduction in ERK MAP kinase activation and prolonged STAT-3 activation after gp130 stimulation. The gp130F759/F759 mice show increased production of Th1-type cytokines and Igs of the IgG2a and IgG2b classes, while these molecules are decreased in the gp130F759/F759 immune system. These results indicate that the SHP-2–mediated or Y759–dependent signals negatively regulate the biological responses elicited by the STAT-3–mediated signals in vivo, and that the balance of positive and negative signals generated through gp130 is skewed or shifted to positive STAT-3 signaling in gp130F759/F759 mice (33). Here we report that gp130F759/F759 mice spontaneously developed arthritis in a manner dependent on lymphocytes accompanied by autoantibody production and T cell abnormalities at ~1 y of age. The gp130F759/F759 mouse is thus a unique animal model in which a point mutation in a cytokine receptor results in a RA-like autoimmune disease.

Materials and Methods

Animals. The establishment of the gp130F759/F759 knock-in mouse line was described previously (33). Heterozygous mice of a 129/C57BL/6 mixed background were intercrossed to obtain homozygous knock-in mice in the F3 generation, which were used for the studies described in this paper except for generating gp130F759/F759 recombination activation gene (RAG)-2−/− mice. gp130F759/F759/RAG-2−/− mouse was generated by crossing gp130F759/F759 mouse with RAG-2–deficient mouse of 129S6/C57BL/6F1 background. To make a F1 generation of RAG-2–deficient mouse in a C57BL/6 and 129S6/SvEv background, we obtained RAG-2–deficient mouse, that had been backcrossed to C57BL/6 eight times, and RAG-2–deficient mouse with a 129S6/SvEv background from M. Itoh (Laboratory of Immunology, Central Institute for Experimental Animals, Kanagawa, Japan) and Taconic, respectively. To obtain lymphocytes from gp130F759/F759 mice, which die as neonates, we reconstituted lethally irradiated mice with liver cells from gp130F759/F759 fetuses that were harvested at 14.5 d after coitus, as described previously (reconstituted mice; reference 33). These mice were kept at the Institute of Experimental Animal Sciences at Osaka University Medical School.

Detection of Igs and Autoantibodies in Sera. To analyze the relationship between the serum antibody levels including autoantibodies, severity of the arthritis, and sex differences, we collected sera from gp130F759/F759 mice with arthritis and gp130F759/F759 mice of both sexes at 11–12 mo old (male gp130F759/F759, n = 14; female gp130F759/F759, n = 17; male gp130F759/F759, n = 8; and female gp130F759/F759, n = 8). Serum levels of total Igs were determined by an isotype-specific ELISA as described previously (34). Rheumatoid factor (RF) for mouse Ig of both IgM and IgG classes, anti-dsDNA, and anti–ssDNA antibodies were measured using commercial ELISA kits (Shibayagi, Japan). An ELISA kit for anti–nRNP antibody was purchased from Alpha Diagnostic. To detect RF, 100 μl of 200-fold diluted serum was added to each well of the ELISA plate precoated with Fc fragments of mouse IgG. The plates were incubated for 2 h and washed three times
with washing buffer. Then, horseradish peroxidase–labeled goat anti–mouse IgG, which was deprived of anti–mouse Fc antibodies by extensive absorption, was added. After 2 h incubation, the plates were washed three times, substrate was added and the absorbance at 450 nm was measured by the microplate reader.

**Clinical Assessment of Arthritis.** The mice were assessed by two independent observers for signs of arthritis: redness, swelling, and restriction of mobility. The severity of the arthritis was based on five signs, which were each assessed bilaterally: (1) swelling of digits of the forelimb; (2) swelling of the footpad; (3) swelling of the ankle; (4) restriction of mobility of the wrist joint; and (5) restriction of mobility of the ankle joint. The severity of the arthritis was graded on a scale of 0–2 for each of the five conditions as follows: 0 (no change); 1 (mild change); and 2 (severe change). The severity score shown in Fig. 1 b is a sum of the scores for the five signs, assessed bilaterally, to give a possible maximum of 20 points for each mouse. The incidence was expressed as the percentage of mice that showed visible arthritis.

**Radiology and Histology.** X-ray photographs of the joints were taken using a SofTEX CMB-2 (SofTEX Co., Ltd.) and Fuji Film. For the histologic examination, joints were fixed in 4% paraformaldehyde, decalcified in 10% EDTA–Na, and embedded in paraffin. Sections were stained with H&E. To detect corresponding cytokine mRNAs by RT-PCR, specific primers and probes reported previously were used (35). The specific primer pairs for FasL and SOCS-3 were as follows: FasL 5′-CGATACGCAGTCTCAAGG-3′ and 5′-GTATTTCCA-3′. PCR reactions were performed in the GeneAmp 5700 Sequence Detection System (Applied Biosystems). The relative amounts of transcripts were normalized to the HPRT transcript.

**FACS® Analysis.** Flow cytometry analysis was performed as described previously (36), and analyzed using a FACS CaliburTM flow cytometer (Becton Dickinson). mAbs used are as follows: FITC-anti-IgM (AM/3); FITC-anti-CD44 (IM7); FITC-anti-CD25 (PC61); FITC-anti-Gr-1 (RB6–8C5); FITC-anti-TCR-α for HY (T3.70, a gift from Y. Takahama, RIKEN Research Center for Allergy and Immunology, Tokyo, Japan); PE-anti-CD28 antibody (clone 37.51; BD Pharmingen); and APC-anti-CD4. Thymocytes were analyzed with a FACSCaliburTM flow cytometer.

**Statistical Analysis.** Statistical analyses for growth responses, numbers of T3.70+CD8+ thymocytes, and SEB-induced clonal deletion were performed by Student’s t test. Serum levels of Igs and autoantibodies in male and female mice were measured using an enzyme-linked immunosorbent assay. The severity scores were analyzed using a Mann-Whitney U test. Correlation between serum autoantibody levels and severity scores was examined using Spearman’s rank correlation. P < 0.05 is thought as significant.

**Results**

**Spontaneous Development of Arthritis in gp130F759/F759 Mice.** By as early as 8 mo, the gp130F759/F759 mice showed swelling and redness of the paws and limited mobility of the ankle or wrist joints (Fig. 1 d). Neither wild-type nor
Representative histology results are shown in Fig. 1, i–p. In the ankle joint of gp130F759/F759 mice, marked proliferation of the synovium with pannus formation accompanied by the infiltration of inflammatory cells, predominantly neutrophils (Fig. 1 j, inset), with fibrin deposits and severe bone destruction were observed (Fig. 1 j, and control, i). At the site of bone erosion, activated osteoclasts, which were identified as TRAP$^{+}$ multinucleated cells, were observed (Fig. 1 i, and control, p). The knees were also affected (Fig. 1 l and control, k). In the mice with rigid ankles and toes, a severe narrowing of joint spaces and ankylosis were observed (Fig. 1 n and control, m).

In radiologic examinations, generalized osteoporosis and marked joint destruction were noted in all limbs. The phalangeal and tarsal bones were destroyed, and prominent bone fusions and reactive sclerotic changes in the tarsal bones were observed (Fig. 1 f, and control, e). In addition, severe joint destruction and ankylosis were also observed in the larger bilateral joints such as the ankles, knees, and hips (Fig. 1 h, and control, g). All these clinical, histologic, and radiologic findings in the gp130F759/F759 mice resemble those of human RA, although some differences were observed, such as the relatively small number of infiltrated lymphocytes and plasma cells in the mice. Quantitative
RT-PCR analysis of cytokine transcripts in the joints of the gp130F759/F759 mice revealed significantly higher transcriptional levels of IL-6 (fold ± SEM: 24 ± 9, P < 0.05) than in controls. Although it was not statistically significant, some cases of arthritis mice showed elevated transcriptional levels of IL-1β (2.4 ± 0.3), IL-10 (3.6 ± 1.0), and TNF-α (1.8 ± 0.9). Thus, the balance of the local cytokine production tended to become skewed toward a proinflammatory status.

Autoantibody Production in gp130F759/F759 Mice. Spontaneous development of RA-like arthritis in gp130F759/F759 mice prompted us to examine autoantibody production of mice with arthritis. At the age of 9 wk, the serum Ig levels of all isotypes in the gp130F759/F759 Igs were similar to those in wild-type littermates (33). However, at 11–12 mo of age, serum Igs were slightly increased in gp130F759/F759 mice with arthritis. The serum levels of IgG1 (average OD ± SEM: male, gp130F759/F759, 0.37 ± 0.01; gp130WT/WT, 0.31 ± 0.02, P < 0.05, female, gp130F759/F759, 0.40 ± 0.01; gp130WT/WT, 0.23 ± 0.02, P < 0.01) and IgA (male, gp130F759/F759, 0.32 ± 0.01; gp130WT/WT, 0.26 ± 0.01, P < 0.01, female, gp130F759/F759, 0.33 ± 0.01; gp130WT/WT, 0.26 ± 0.02, P < 0.01) were increased in both male and female gp130F759/F759. IgG2a in female (gp130F759/F759, 0.24 ± 0.01; gp130WT/WT, 0.19 ± 0.01, P < 0.01) and IgG2b in male (gp130F759/F759, 0.38 ± 0.00; gp130WT/WT, 0.32 ± 0.02, P < 0.05) were a little bit increased.

There was no significant difference in the increase of serum Ig levels between male and female gp130F759/F759 mice (unpublished data) except for IgG1 (male, 0.37 ± 0.01; female, 0.40 ± 0.01, P < 0.05). More importantly, the 11–12-mo-old gp130F759/F759 mice produced significantly higher levels of autoantibodies, such as RF of the IgG class, anti-ssDNA antibody (0.33 ± 0.14, and 1.40 ± 0.27, P < 0.01), anti-dsDNA antibody (0.20 ± 0.08 and 0.83 ± 0.18, P < 0.01) and anti-nRNP antibody (0.88 ± 0.11, and 1.49 ± 0.16, P < 0.01) (Fig. 2). These results suggest that the abnormal gp130 signals broke the homeostasis of the immune system and elicited autoimmunity. However, there were neither proteinuria nor pathological changes in the kidney (unpublished data).

Then, we statistically examined the correlation between the severity and the level of each autoantibody in females and males. However, there was no correlation between the level of each autoantibody and severity score. Some gp130F759/F759 mice had sporadic elevation of the serum IgM-RF, being not correlated with severity score (unpublished data). We also examined the correlation between the level of each autoantibody in males and females: IgG-RF versus IgM-RF, IgG-RF versus anti-ssDNA, IgG-RF versus anti-dsDNA, IgG-RF versus anti-nRNP, anti-ssDNA versus anti-dsDNA, and anti-dsDNA versus anti-nRNP. Among them, there were correlations in IgG-RF versus anti-dsDNA (r = 0.54, P < 0.05), IgG-RF versus anti-nRNP (r = 0.67, P < 0.01), and anti-dsDNA versus anti-nRNP (r = 0.84, P < 0.01) in females. In males, only anti-ssDNA versus anti-dsDNA showed correlation (r = 0.99, P < 0.01). These results suggest that the hormonal environment affects the autoimmunity in gp130F759/F759 mice.

Increase in Activated T Cells and Myeloid Cells in the Lymphoid Organs of gp130F759/F759 Mice. To understand the involvement of the immune system in the arthritis of the gp130F759/F759 mice, we first analyzed the cell populations in their lymphoid organs compared with those of their gp130F759/WT littermates. Analysis of the thymus of the diseased mice revealed a lower frequency of CD4/CD8 double-positive cells and a higher frequency of CD4/CD8 double-negative cells and CD4 or CD8 single-positive cells than their gp130F759/WT littermates (Fig. 3 a). The population of CD4/CD8 double-negative cells included IgMα

Figure 2. Autoantibody production in gp130F759/F759 mice. Serum from 11–12-mo-old gp130F759/F759 mice with arthritis (n = 31; 14 male, 17 female) and their wild-type littermates (n = 16; 8 male, 8 female) was assayed for the levels of autoantibodies by ELISA. White and black circles represent the serum levels of antibody in individual gp130WT/WT and gp130F759/F759mouse, respectively. Horizontal bars represent the mean values of each group. Asterisks indicate significant differences by Mann-Whitney U test (*P < 0.05; **P < 0.01).
IgDhi conventional B cells, CD45R+Syndecan-1+ plasma cells, and CD11b+(Gr-1+ and Gr-1−) cells (Fig. 3 b and unpublished data). These abnormalities of thymic populations expressing CD4 or CD8 were present to various degrees in 80% (16 out of 20) of the mice with arthritis. The lower frequency of CD4/CD8 double-positive cells and the higher frequency of CD4 or CD8 single-positive cells in the thymus were unlikely to be due to the stress experienced by the mice with arthritis, because these differences began to be observed in gp130F759/F759 mice as young as 18 wk old, before the onset of the arthritis (unpublished data).

In the lymph nodes of the gp130F759/F759 mice with arthritis, the numbers of myeloid cells, especially Gr-1+ CD11b+ cells (Fig. 3 c), were higher to various degrees. Correlating with the level of increase in the number of neutrophils, B cells were relatively fewer in the mice with arthritis (unpublished data). The number of B-1 cells was not higher in the spleens of the mice with arthritis. Although the ratio of CD4 to CD8 T cells showed no consistent changes, the CD62L+CD44− naive T cell subset was almost completely absent in the populations of both CD4+ and CD8+ T cells (Fig. 3 e and f). Instead, the numbers of CD62L−CD44+ memory/activated T cells and CD62L+CD44+ T cells were higher in the CD4+ and CD8+ T cell populations, respectively. The activated status of the T cells in the arthritic mice was further indicated by the higher numbers of CD4+ T cells expressing CD69 or CD25, which are early activation markers (Fig. 3 d). The number of CD8+ T cells expressing CD69 or CD25, however, was rarely higher in the arthritic mice (unpublished data). These differences were also observed in the spleen, indicating that the peripheral T cells, especially the CD4+ T cells, were chronically activated in the gp130F759/F759 mice with arthritis.

Hyperresponsiveness of Thymocytes and Lymph Node T Cells in Young gp130F759/F759 Mice. The flow cytometry analysis indicated no abnormalities in the major population of surface markers of lymphoid cells from gp130F759/F759 mice younger than 12-wk-old (Fig. 4, a and b, top). However, the anti-CD3 antibody-induced T cell growth responses were much higher in the thymocytes and lymph node cells of young gp130F759/F759 mice than in wild-type mice (Fig. 4, a and b, bottom), indicating that the functional abnormality of the T cells was present in the gp130F759/F759 mice even before disease onset.

Impairment of Thymic Negative Selection and Clonal Deletion in the Peripheral T Cells of gp130F759/F759 Mice In Vivo. Both central and peripheral tolerances play crucial roles in maintaining self-tolerance (37, 38). We crossed gp130F759/F759 mice with anti–HY-TCR transgenic mice to examine the effect of the gp130F759/F759 mutation on thymic selection (39). In the female anti–HY-TCR gp130F759/F759 thymocytes, the frequency of CD8+ cells was similar to anti–HY-TCR gp130WT/WT thymocytes (Fig. 5 a, top), indicating that positive selection was not impaired. In the male anti–HY-TCR gp130F759/F759 thymocytes, a severe reduction in total cell number and CD4/CD8 double-positive cells was observed, similar to that seen in anti–HY-TCR gp130WT/WT thymocytes, but the number of CD8+ thymocytes (Fig. 5 a, left and right bottom; in the upper left quadrant and Fig. 5 b), which are thought to be the thymocytes that have escaped negative selection (40), was higher in gp130F759/F759 mice, indicating that the negative selection in gp130F759/F759 mice was impaired to some ex-
tent. Then, we examined the effects of the Y759 mutation on the peripheral clonal deletion of activated T cells. For this study we injected a superantigen, SEB, into the mice (41). The frequency of Vβ8-positive peripheral blood T cells that had been specifically activated by SEB was decreased by deletion in wild-type mice injected with SEB. On the other hand, the frequency of Vβ6+CD4+ T cells in the gp130F759/F759 mice was not reduced (Fig. 6 a). The frequency of Vβ6+CD4+ T cells that had not been activated by SEB showed no reduction in either gp130F759/F759 or wild-type mice (Fig. 6 b). These results indicated that both thymic selection and clonal deletion of peripheral T cells were impaired in the gp130F759/F759 mice.

**Inhibitory Effect of IL-6 on Fas Ligand Expression Was Augmented in gp130F759/F759 T Cells During AICD.** The accumulation of activated T cells in the lymphoid organs and resistance to SEB-induced peripheral clonal deletion in gp130F759/F759 mice suggested that AICD is impaired in gp130F759/F759 T cells. Anti-CD3 antibody induced similar levels of AICD (≈50%) in both gp130F759/F759 and control T cells. In the presence of IL-6, anti-CD3–induced AICD was inhibited and this inhibitory effect was, however, higher in gp130F759/F759 T cells than wild-type T cells (Fig. 7 a). To identify molecules responsible for the IL-6–dependent resistance to AICD, we first examined the expression of molecules related to activation and apoptosis in the primed T cells. The expression levels of Fas, CD25, CD69, Bcl-2, and Bcl-X before and after the induction of AICD were similar in wild-type and gp130F759/F759 T cells (unpublished data). The expression of FasL on gp130F759/F759 T cells stimulated with the anti-CD3 antibody alone was similar to wild-type T cells. Addition of IL-6 to anti-CD3 stimulation only slightly decreased the frequency of FasL-positive cells (22–18%) in wild-type T cells (Fig. 7 b). This negative effect of IL-6 was more evident in gp130F759/F759 T cells (20–10%) than in wild-type T cells. A similar negative effect of IL-6 on FasL expression was observed at the transcription level (Fig. 7 c), but IL-6 had no effect on the anti-CD3–induced upregulation of IL-2 mRNA (unpublished data). To understand the molecular mechanisms of the negative effect of IL-6 on FasL expression, we examined whether the gp130-mediated signals in gp130F759/F759 T cells were abnormal in any way.

As shown in Fig. 7 d, the IL-6–induced tyrosine-phosphorylation of JAK-1 and STAT-3 was prolonged, compared with wild-type T cells, consistent with the previous results observed in gp130F759/F759 T cells during AICD. Therefore, our results suggested that AICD is impaired in gp130F759/F759 T cells. Anti-CD3 antibody induced similar levels of AICD (≈50%) in both gp130F759/F759 and control T cells. In the presence of IL-6, anti-CD3–induced AICD was inhibited and this inhibitory effect was, however, higher in gp130F759/F759 T cells than wild-type T cells (Fig. 7 a). To identify molecules responsible for the IL-6–dependent resistance to AICD, we first examined the expression of molecules related to activation and apoptosis in the primed T cells. The expression levels of Fas, CD25, CD69, Bcl-2, and Bcl-X before and after the induction of AICD were similar in wild-type and gp130F759/F759 T cells (unpublished data). The expression of FasL on gp130F759/F759 T cells stimulated with the anti-CD3 antibody alone was similar to wild-type T cells. Addition of IL-6 to anti-CD3 stimulation only slightly decreased the frequency of FasL-positive cells (22–18%) in wild-type T cells (Fig. 7 b). This negative effect of IL-6 was more evident in gp130F759/F759 T cells (20–10%) than in wild-type T cells. A similar negative effect of IL-6 on FasL expression was observed at the transcription level (Fig. 7 c), but IL-6 had no effect on the anti-CD3–induced upregulation of IL-2 mRNA (unpublished data). To understand the molecular mechanisms of the negative effect of IL-6 on FasL expression, we examined whether the gp130-mediated signals in gp130F759/F759 T cells were abnormal in any way.

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As shown in Fig. 7 d, the IL-6–induced tyrosine-phosphorylation of JAK-1 and STAT-3 was prolonged, compared with wild-type T cells, consistent with the previous results observed in gp130F759/F759 fibroblasts (33). Furthermore, RT-PCR analysis revealed that IL-6 induced SOCS-3 but not SOCS-1 mRNA in the activated gp130F759/F759 T cells, and that this induction of SOCS-3 in the activated gp130F759/F759 T cells was enhanced as compared with gp130WT/WT T cells, probably reflecting the increased STAT-3 activation (Fig. 7 e, and unpublished data). These results raised the possibility that a prolonged activation of STAT-3 might interfere with FasL expression. To test this possibility, we used T cells purified from recipient mice (Materials and Methods) with gp130FXXQ/FXXQ fetal liver cells, which express gp130 mutated at the four tyrosine residues required for gp130–mediated STAT-3 activation. As shown in Fig. 7 d, IL-6 did not induce tyro-
sine-phosphorylation of STAT-3 in these T cells. Furthermore, the negative effect of IL-6 on AICD and FasL expression was not observed in \( \text{gp130}^{\text{FFXQ}/\text{FFXQ}} \) T cells (Fig. 6, a–c). Taken together, these results strongly suggest that gp130-mediated STAT-3 activation plays a negative role in the anti-CD3-induced upregulation of FasL in activated T cells.

Dependence of Arthritis Development on Lymphocytes. All results described above showed that there were several abnormalities of immune responses in \( \text{gp130}^{\text{F759}/\text{F759}} \) mice, suggesting that abnormal immunity is critically involved in arthritis development. To clarify this issue, we investigated whether lymphocytes are actually required for the disease. To address this issue, we crossed \( \text{gp130}^{\text{F759}/\text{F759}} \) mice with \( \text{RAG-2}^{-/-} \) mice to generate \( \text{gp130}^{\text{F759}/\text{F759}} \text{RAG-2}^{-/-} \) double mutant animals as described in Materials and Methods. 81% of the \( \text{gp130}^{\text{F759}/\text{F759}} \text{RAG-2}^{-/-} \) mutant mice (13 of 16) had developed arthritis (average score \( \pm \text{SEM} \), 40.6 \( \pm \) 2.7) at 14 mo old. However, the \( \text{gp130}^{\text{F759}/\text{F759}} \text{RAG-2}^{-/-} \) mice did not develop arthritis (\( n = 15 \)), except for one case showing only a mild, transient restriction of joints (Fig. 8). These results clearly establish that lymphocytes are essential for the development of the arthritis in the \( \text{gp130}^{\text{F759}/\text{F759}} \) mice.

**Discussion**

Here we showed that a point mutation of Y759 in \( \text{gp130} \) in mice caused spontaneous development of an age-dependent RA-like joint disease accompanied by autoantibody production and T cell abnormalities, including impairment of peripheral clonal deletion. Most importantly, lymphocytes were required for disease development. The arthritis in \( \text{gp130}^{\text{F759}/\text{F759}} \) mice developed in middle age and its clinical course was chronic and progressive. It started...
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with mild swelling and redness of the paws, and joint mobility decreased thereafter. The larger joints were affected symmetrically, and the joints eventually became ankylosic. Radiologic analysis of the affected joints revealed the characteristics of advanced human RA. Histologic examination showed leukocytes infiltrating the joint space, hyperplasia of the synovium with pannus formation, destruction of the cartilage and bone, and bony ankylosis, all of which resemble characteristics of human RA.

Foci of lymphocytes and plasma cells were scattered around periarticular regions, although the degree of infiltration of these cells was less than in human RA. The transcription of proinflammatory cytokines, in particular IL-6, in the joints was increased. Although the infiltration of lymphocytes was not marked around the joint tissues, the involvement of an immunological abnormality in the pathophysiology of the arthritis was indicated since the development of arthritis was totally dependent on lymphocytes: introduction of the \( \text{gp130}^{F759/759} \) mutation into \( \text{RAG-2}^{-/-} \) mice did not cause arthritis. This is in sharp contrast to the arthritis that develops in TNF AU-rich elements-deficient mice, which is independent of lymphocytes (9). Consistent with this notion, production of a variety of autoantibodies was observed in the diseased mice. The involvement of autoantibody in RA or experimentally induced arthritis model has been recently reconsidered (2, 7).

However, we did not observe any correlation between the level of any of autoantibody and severity, suggesting...
that B cell response may be less important than T cell response. In line with the hypothesis that T cells play roles in the disease we observed several abnormalities of T cells. In the thymus of mice with arthritis, the frequency of CD4/CD8 double-positive cells decreased and that of CD4 or CD8 single-positive cells increased. In the lymph nodes of gp130F759/F759 mice with arthritis, a decrease in naive CD4+ and CD8+ T cells, and an increase in memory/activated CD4+ T cells were observed. These abnormal T cell populations appeared in young mice before the onset of the disease. Functionally, the in vitro hyperresponsiveness of T cells in the thymus and lymph nodes was also observed before disease onset. The impairment of both thymic negative selection and peripheral clonal deletion suggests that self-tolerance is disrupted in gp130F759/F759 mice. Taken together, all results suggest that T cells may play critical roles in the development of arthritis.

Ernst et al. reported that the knock-in mouse gp130STAT3 STAT3 displays impaired acute phase and immune responses and develops severe gastrointestinal ulceration and joint disease (42). However, the joint disease that develops in Ernst’s mouse is quite different from RA and arthritis. The impairment of FasL expression. Alternatively SOCS-3 may interfere T cell receptor-mediated signaling involved in Fasl induction since IL-6–mediated SOCS-3 induction was enhanced in gp130F759/F759 T cells as compared with wild-type T cells. In any case, it is evident that gp130F759/F759 T cells showed suppressed FasL expression in the presence of IL-6 in a manner dependent on the tyrosine residues of gp130 that are required for STAT-3 activation. Regardless of the precise mechanism, it is reasonable to speculate that resistance of T cells in gp130F759/F759 mice to clonal deletion in vivo is also involved in the development of autoimmune disease by the breakdown of self-tolerance. An intriguing possibility is that very mild impairment of both central and peripheral tolerance is required for the development of age-dependent autoimmune disease in gp130F759/F759 mice. Study of the signal transduction pathways that are initiated through gp130 has shown that a single cytokine receptor can simultaneously transduce multiple, contradictory signals, leading to a variety of responses, such as growth and differentiation, and that the balance of the contradictory signals determines the biological output of a given cytokine (24).

The fact that selective disruption of gp130 signals dependent on Y759, which augments gp130-mediated STAT-3 activation (33), resulted in joint disease with autoimmunity, provides an intriguing, unique case where the disruption of the balance of signals transduced from one cytokine receptor may cause autoimmune disease. It is also possible that lack of negative feedback mechanisms through SOCS-3 may be involved in enhanced STAT-3 activation through gp130 and the generation of the disease, since Y759 is shown to be required for the negative effect of SOCS-3 (31, 32). Another possibility is that enhanced induction of SOCS-3 through gp130 may modulate signaling by other cytokines.

Although we clearly showed that lymphocytes are required for disease development, it is also possible that abnormal gp130-mediated signals generated in other cells...
function with lymphocytes to cause diseases because gp130 is expressed in a variety of cells, including macrophages, granulocytes, dendritic cells, fibroblasts, epithelial cells, endothelial cells, synovial cells, and osteoclasts. Among them, granulocytes infiltrating in the synovium may play an important role in the course of arthritis.

Taken together, our results show that the gp130F759/F759 mutant mouse is a new animal model of autoimmune arthritis and is unique in demonstrating that a point mutation of a cytokine receptor can trigger complicated immunological and inflammatory processes, leading to the generation of autoimmune disease.

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